Amendments to the Specification:

Please amend the specification as follows:

Please insert the enclosed sequence listing into the specification.

At page 7, lines, line 2-23, please delete the entire section following the heading "Brief Description of the Drawings" and insert the following rewritten section in place thereof:

FIG. 1, comprising FIG. 1A through FIG. 1F, is a series of images demonstrating that β-lactam antibiotics are inducers of EAAT2 protein based on a screen of 1040 FDA approved drugs. FIG. 1A shows spinal cord cultures incubated with test compound; FIG. 1B is a sample slot blot from tissue homogenates; FIG. 1C illustrates a representative screening slot blot; FIG. 1D illustrates screening results of a library of test compounds; FIG. 1E is an illustration of expression results from treatment with various compounds categorized by classes; FIG. 1F shows a dose-response analysis of EAAT2 expression for ceftriaxone.

FIG. 2, comprising FIG. 2A through FIG. 2G, is a series of images demonstrating the generation of promoter reporter transgenic mice. FIG. 2A-2E illustrate expression of EAAT2 promoter fragments in mouse brain at two weeks, and widespread expression of the reported in astrocytes throughout the brain parenchyma. FIG. 2F shows astrocytes from EAAT1 promoter reporter, and FIG. 2G shows cortical expression of EAAT1 Bac-eGFP reporter, in transgenic mice.

FIG. 3, comprising FIG. 3A and FIG. 3B, is a series of images demonstrating that β-lactams activate EAAT2 promoter. FIG. 3A shows activation (by compound class) of EAAT2 promoter by various test compounds in Cos7 cells and FIG. 3B shows activation (by compound class) of EAAT2 promoter in human astrocytes transfected with the EAA52 promoter-eGFP reporter. β-lactam antibiotics (10μM) markedly activate the EAAT2 promoter, while controls such as glutamate have no effect. The known activator, dibutyryl cyclic AMP, has a consistent, but smaller effect.

FIG. 4, comprising FIG. 4A though FIG. 4D, demonstrates that ceftriaxone induces expression of GLT1 and GLT1b, but not other proteins, in vivo. Rats were injected (ip) daily for 5 days with ceftriaxone (200mg/kg) - a dose known to produce low micromolar brain concentrations. FIG. 4A is a western blot of ceftriaxone effect on GLT-1 and GLT-1B expression in the hippocampal; FIG. 4B illustrates the effect of ceftriaxone on GLT-1 and GLT-1B expression in the hippocampal; FIG. 4C is a western blot of ceftriaxone effect on GLAST, EAAC1 and EAAT4 expression; FIG. 4C illustrates the effect of various antibiotics on glutamate transport.

FIG. 5 demonstrates that β-lactam antibiotic ceftriaxone and penicillin administration leads to a functional increase in glutamate transport. Daily treatment with ceftriaxone or penicillin (200mg/kg, 5 days) not only increased GLT1 protein, but also increased GLT1-mediated glutamate transport, as determined by ^{3}H-glutamate transport assays (in the presence/absence of dihydrokainate- to measure GLT1-specific transport). It was observed that the non-β-lactam antibiotic vancomycin, did not increase glutamate transport activity.

FIG. 6, comprising FIG. 6A through FIG. 6D, demonstrates neuroprotection by ceftriaxone.. FIG. 6A illustrates the effect of ceftriaxone on ischemic tolerance. It was observed that oxygen glucose deprivation (OGD) of cortical neurons lead to reliable cell death; while preconditioning with brief OGD was protective. Similar protection was afforded by ceftriaxone (1μM) pretreatment. FIG. 6B illustrates the effect of ceftriaxone on motor neuron degeneration. It was observed that ceftriaxone prevented motor neuron degeneration in vitro. Chronic treatment of spinal cord organotypic cultures with the glutamate transport inhibitor threo-hydroxyaspartate lead to loss of >50% neurons. Co-treatment with ceftriaxone prevented this excitotoxic loss of motor neurons. FIG. 6C illustrates the effect of ceftriaxone on grip strength (in vivo model); FIG. 6 D illustrates the effect of ceftriaxone on survival in G93A (SOD1 ALS mice. It was observed that ceftriaxone therapy (200mg/kg daily ip) delayed loss of muscle strength in G93A SOD1 mice. Therapy was initiated at disease onset (approx 12 wks age). Asterisks indicate significant difference from saline controls ($P<0.05$) at each time point.

At page 26, immediately before line 22, please insert the following new paragraph:

Figure 1 depicts the results from a screen of 1040 FDA approved drugs. The screen revealed that β-lactam antibiotics serve as inducers of EAAT2 protein. Briefly, the screen includes incubating spinal cord cultures with compounds for 3 days (Figure 1A). Figure 1B depicts a sample slot blot from tissue homogenates as a control to validate the increased EAAT2 expression seen with increasing controls known to occur with dibutyryl cyclic AMP, GDNF or the neuroimmunophilin GP1-1046. All three compounds (dbcAMP, GDNF and GP1-1046) induced a large increase in EAAT2 expression, after three days in culture. Figure 1C depicts a typical data slot blot of EAAT2 protein from an early screen of various agents. Evey blot included control-untreated tissue and a positive control from panel B. Figure 1D depicts screening results from the NIH-NINDS Custom Collection screen of 1040 FDA approved compounds. Height of the bars reflects increased EAAT2 protein expression relative to untreated controls. Each blot included at least one untreated control and one known positive control. Figure 1E depicts β-lactam antibiotics were highly represented in the most potent compounds. These compounds were able to increase EAAT2 protein expression up to 7 fold compared to untreated control cultures after a 7 days chronic treatment. Figure 1F depicts a dose response analysis for ceftriaxone, revealing EC_{50}3.5μM for EAAT2 expression.

Please replace the paragraph at page 26, lines 21-33 and page 27, lines 1-4, with the following amended paragraph:

Bioassay Method see **Figure [[2]]** 1 for assay description summary.

Organotypic Spinal Cord. Spinal cord organotypic cultures have been described by us in detail in the past. Rothstein JD et al., *N. Engl. J. Med.* 1992: 236:1464-1468. Briefly, 300 um sections of rat lumbar spinal cord, from postnatal day 8-10 rat pups, are placed on Millipore Millicell CM semipermeable membranes. Each well contains 5 slices (**Figure [[2A]]** **1A**). Fifty-100 cultures can be prepared weekly. Each drug (10-100 μM) was added for 3 - 7 days, along with cell culture medium/serum. Cultures were harvested and 5-50 μg of tissue was applied to

slot blot apparatus for detection of EAAT2 by standard Western blotting/chemiluminescence methods described in the past. Kuncl RW et al., Motor neuron diseases. In: Asbury AK et al., editors. *Diseases of the Nervous System.* 2 ed. Philadelphia: W.B. Saunders, 1992; 1179-1208; Rothstein JD et al. *Neuron* 1994; 13:713-725. All antipeptide antibodies were affinity purified and highly specific for transporter subtypes. A typical slot-blot analysis, is shown in **Figure [[2B,C]] 1B, 1C**. By this method, increases greater than 50% of expressed protein can be detected. For each antibody slot blot, the homogenates used are expected to be within the linear range for antibody detection, based on prior standard curves.

Please replace the paragraph at page 27, lines 5-15, with the following amended paragraph:

Screening Library–Assay Design. The library of compounds for these first studies was the NINDS Custom Collection from Microsource Discovery. The library is composed of 1040 compounds in 96 well plates, that also concluded positive control for transporter synthesis (dibutyryl cyclic AMP [dbcAMP], GDNF). These compounds (dbcAMP, GDNF, GPI-1046) induced a large increase in EAAT2 expression after 3 days in culture. The library is a unique collection of known bioactive compounds that permit the simultaneous evaluation of hundreds of marketed drugs and biochemical standards. Each compound was studied at a final concentration of 10-100μM. All assays were performed in duplicate. Every blot included control - untreated tissue and a positive control, such as those identified above. A typical slot blot is shown in **Figure [[2C]] 1C**.

Please replace the paragraph at page 27, lines 16-22, with the following amended paragraph:

Data Analysis. All blots were analyzed by laser densitometry (BioRad Image Quant) and the duplicate points were averaged. The complete result dataset from the 1040 compounds is shown in **Figure [[2D]] 1D**. Each blot included a positive control standard (e.g. dbcAMP) and a

negative control standard (e.g. serum, DMSO). Data was kept in Excel Spreadsheets, using a numerical/text coding system. All positive drugs (positive defined as at least a 50% increase[[d]] in protein expression) were re-evaluated.

Please replace the paragraph at page 27, lines 23-33, with the following amended paragraph:

RESULTS: Screened Drugs Can Increase EAAT2 In vitro. After screening 1040 compounds, we were able to identify more than 10 related compounds capable of increasing EAAT2 protein levels by 3.5 to 7 fold (see **Figure [[2E]] <u>1E</u>**). In total, we identified 80 compounds capable of increasing EAAT2 by 2 fold or more in the first screen. Of that list, β-lactam antibiotics were overly represented and were the most common structural motif observed in all compounds-- 15 different beta lactam antibiotics, including penicillin and derivatives, were active. As shown in **Figure [[2E]] <u>1E</u>**, these β-lactams were all capable of increased EAAT2 protein expression. A follow-up dose response analysis (**Figure [[2F]] <u>1F</u>**) revealed [[and]] EC_{50} for protein expression for ceftriaxone of 3.5 μM.

Please replace the paragraph at page 28, lines 2-14, with the following amended paragraph:

Generation of E2P-eGFP and BAC-EAAT1-eGFP Transgenic mice (Figure [[3]] <u>2</u>). To provide screening cell lines for the assays we have now successfully generated two transgenic mice that express the EAAT2 promoter fragment (E2P) or the full length EAAT1 promoter (Bac-EAAT1). As shown in **Figure [[3]] <u>2</u>** we have generated E2P transgenic mice that demonstrate widespread expression of the EAAT2 promoter reported in the CNS. ~~Similar~~ S<u>imilarly,</u> we generated Bac-EAAt1 mice and expressing cells. Recently the Heintz group also generated an EAAT1Bac-reporter based mouse based on a similar Bac construct used in our own mice.

Please replace the paragraph at page 28, lines 30-33, and page 29, lines 1-4, with the following amended paragraph:

RESULTS: Identification of EAAT2 Promoter activating compounds (Figure [[4]] 3) From the original NINDS screen, we identified numerous β lactam compounds capable of potently activating EAAT2 promoter. As shown in **Figure [[4]]** 3, most β-lactams were able to increase EAAT promoter- far more than the known positive control, dibutyrl cyclic AMP. All compounds were active at a pharmacologically relevant concentration of 1-10uM—a concentration range that these compounds can be found in the CNS after standard anti-bacterial therapy (e.g. ceftriaxone).

Please replace the paragraph at page 29, lines 9-20, with the following amended paragraph:

Ceftriaxone Increases Brain GLT1 level (Figure [[5]] 4). To determine if a drug identified herein could actually induce EAAT expression in vivo, we administered ceftriaxone to rats (n=5) (and mice, n=3) daily. Ceftriaxone was administered at a dose known to lead to CNS levels, 200 mg/kg ip. After 5 days of chronic daily administration animals were sacrificed and brain tissue harvested. As shown in **Figure [[5A,B]] 4A, 4B,** ceftriaxone therapy ~~lead~~ led to 3 fold increase in brain GLT1 levels, as well as its normal splice product, GLT1b. This increase is comparable to the promoter activation results seen in vitro (**Figure [[4]]** 5). Western blots for the astroglial glutamate transporter GLAST as well as the two neuronal glutamate transporters, EAAC1 and EAAT4, showed no alteration in transporter expression after ceftriaxone therapy (**Figure [[5C,D]] 4C, 4D).** Similarly, the constitutive protein, actin, was unchanged by ceftriaxone administration (**Figure [[5A,C]] 4A, 4C**).

Please replace the paragraph at page 29, lines 28-33, and page 30, lines 1-2, with the following amended paragraph:

In Vitro Model of Ischemia-Oxygen glucose deprivation (Figure [[7A]] 6A). The in vitro model of oxygen glucose deprivation (OGD) is a well known and well accepted model of acute neural injury. In our in-vitro model of ischemia, one hour of oxygen deprivation (OGD) is lethal to cultured neurons, with toxicity known to involve excess glutamate. However, when these cultures are preconditioned 24 hours prior to the lethal condition with transient OGD (5 minutes), there is a dramatic and robust resistance of neurons to cell death. The data indicate that this neuroprotection may be due, in part, to increased expression of GLT1.

Please replace the paragraph at page 30, lines 23-34, with the following amended paragraph:

Ceftriaxone Neuroprotection. Baseline neuronal death in the cultures is 14% as shown in the no treatment column (NT) of **Figure [[7A]]** 6A. Data are presented as average neuronal death in separate wells of one experiment. 1 μM Ceftriaxone, when added for 48 hours in these cultures, does not increase the baseline cell death (NT + Ceftriaxone). When cultures are subjected to 1 hour OGD, neuronal cell death, as expected, increases dramatically to approximately 50%. When cultures are preconditioned with 5 minutes of OGD 24 hours prior to 1 hour OGD, percent cell death is comparable to no treatment condition, indicating ischemic tolerance of neurons in this condition. This is the well known phenomenon of ischemic tolerance. Importantly, 1μM Ceftriaxone, when added 48 hours prior to 1 hour OGD, also protects neurons from cell death, reducing the percentage of neuronal cell death from 50% to 20% (similar to ischemic tolerance neuroprotection). Acute administration of ceftrixone was not protective. Comparable neuroprotection was seen with the betalactam antibiotic cefuroxime. Thus, ceftriaxone pretreatment appears to prevent neuronal death in ischemic tolerance.

Please replace the paragraph at page 31, lines 6-18, with the following amended paragraph:

In vitro model of chronic motor neurodegeneration (Figure [[7B]] 6B). A model of chronic neurodegeneration was used, based on the blockade of glutamate transport in spinal cord organotypic cultures, with the non specific inhibitor threo-hytdroyxaspartate (THA) or TBOA. Chronic incubation of cultures with THA (or TBOA) leads to chronic increase in extra cellular glutamate and subsequent slow death of motor neurons (over 4 weeks). The organotypic spinal cord culture model was developed to study aspects of glutamate-mediated toxicity (and therapy). It has been useful in pre-clinical drug identification (including- riluzole- the only FDA approved drug for ALS, and more recently- celecoxib). Increased expression of glutamate transporter GLT1, by genetic over expression (e. g. transfection or transgenic over expression), in this system, can prevent motor neuron death (not shown) and neuronal death in transgenic animals. Guo H., et al. *Hum Mol Genet.* 2003; 12: 2519-2532.

Please replace the paragraph at page 32, lines 3-10, with the following amended paragraph:

Neural Protection by ceftriaxone. As shown in **Figure [[7B]]** **6B**, ceftriaxone treatment prevented motor neurons loss in a dose dependent manner. As shown in the previous Examples, this concentration of ceftriaxone increases GLT1 protein and function by at least 3 fold. Importantly, the concentrations used in these studies are within the range attainable with oral/parenteral administration of ceftriaxone (1-4 grams/day). Notably, neuroprotection cannot be seen in cultures prepared from GLT-1 null mice (not shown). Similar neuroprotective effects were seen with penicillin, but not vancomycin, which did not alter GLT1 levels.

Please replace the paragraph at page 32, lines 11-21, with the following amended paragraph:

In Vivo Neuroprotection-Effect of ceftriaxone on onset and progression of motor neuron disease in the G93A SOD1 Mouse. (Figure [[7C,D]] 6C, 6D). To determine if ceftriaxone could alter neurodegeneration in a disease model that involves altered expression of glutamate transporters we treated G93A SOD1 mice with ceftriaxone. Numerous studies have documented a contributory role for excess glutamate in this mouse model-and role for modulating glutamate receptors or transporters in neuroprotective strategies. Guo H., et al. *Hum Mol Genet.* 2003; 12: 2519-2532. Modest over expression- by a transgenic approach- can alter disease onset and/or survival. Furthermore, recent studies suggest that late administration of drugs, e. g. at time of disease onset, may be more therapeutically relevant.

Please replace the paragraph at page 32, lines 27-33, and page 33, lines 1-4, with the following amended paragraph:

Ceftriaxone delays loss of Grip Strength and Increases survival. As shown in **Figure [[7C]] 6C**, ceftriaxone treatment significantly delayed loss of muscle strength. This effect was observed within 7 days after treatment, and persisted for 4 weeks; by 18 weeks of age the strength preservation was lost. In a similar manner, the drug also increased over all survival of the mice by about 7-10 days **(Figure [[7D]] 6D)**. Although this effect is relatively small, the drug was given at the time of disease onset, and thus, even a small effect may have clinical significance. When the same dose of drug was administered somewhat earlier, at 42 days of age, survival was also increased, although not significantly better than late delivery at 90 days of age. The lack of greater efficacy when given earlier would be consistent with the observation that the loss of GLT1 expression does not begin to occur until around 90 days in this model.